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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/249,543	02/12/1999	THOMAS C. EVANS	NEB-154	1052

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EXAMINER
MOORE, WILLIAM W

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 09/19/2002

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/249,543	EVANS ET AL.	
	Examiner	Art Unit	
	William W. Moore	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

P riod for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 July 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-61* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *22,23,29 and 30* is/are allowed.

6)☒ Claim(s) *1-4,6-12,14-21, 24, 25,27,28,31-45 and 47-61* is/are rejected.

7)☒ Claim(s) *5,13,26 and 46* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Response to Amendment

Applicant's Amendment C, Paper No. 11 filed July 1, 2002, has been entered amending claims 1-3 and introducing the new claims 31-61, thus claims 1-61 are pending herein. Applicant's comments at pages 8-10 of Paper No. 11 concerning the interview conducted June 20, 2002, are accurate in relating the agreement therein that application of prior art chemical syntheses to generate accepting peptides with a carboxyl-terminal thioester, is inappropriate in rejecting claims that describe a disclosed invention. The amendments of Paper No. 11 remove the basis for rejections of record of claims 1 and 15 under 35 U.S.C. §102 but prior art rejections of claims 1 and 15 under 35 U.S.C. §103(a) are maintained where claims 1 and 15 have a scope that extends beyond a disclosed invention to embrace the suggestions found in teachings of, e.g., Canne et al., Tam, Kent et al., and Offord et al. Rejections of claims 1 and 15 over Evans et al. and Severinov under 35 U.S.C. §102(a) were withdrawn in Paper No. 10 mailed April 23, 2002, in view of Dr. Evans' Declaration under 37 CFR 1.131 filed with Paper No. 9 on February 1, 2002, and documentary evidence attached thereto. A rejection of record of claims 24 and 28 under 35 U.S.C. §102(a) is addressed by a declaration under 37 CFR 1.132 by Dr. Shaorong Chong, Paper No. 12 filed July 1, 2002. The Declaration follows a Petition to amend inventorship under 37 CFR 1.48(a), Paper No. 8, filed February 1, 2002, and granted in Paper No. 10, whereby Dr. Chong became the third co-inventor named in the application. The rejection of claims 24 and 28 over Chong et al., 1998, **The Journal of Biological Chemistry**, Vol. 273, pages 10567-10577, is withdrawn because Dr. Chong states that authors Chong and Xu are co-inventors herein and that the work of the other two co-authors was performed under the direct supervision of Dr. Chong, thus the publication is unavailable as prior art.

The specification discloses accepting, or target, polypeptides that are expressed as amino-proximal exteins in extein-intein fusions in transformed host cells. The accepting, or target, polypeptides then acquire a carboxyl-terminal thioester after *in vitro* cleavage by a thiol reagent at the extein-intein junction and provide this carboxyl-terminal thioester for a nucleophilic attack by the amino-terminal cysteine of a ligation partner polypeptide or peptide. The extein-intein fusion must be isolated from the host cell in which it is expressed for *in vitro* cleavage by the thiol reagent.

The thiol reagent is, in turn, required in Applicant's disclosed method to cleave the target or accepting polypeptide from the extein-intein fusion because there is either no other extein flanking the intein to initiate splicing or the intein is altered, see pages 11-17 of the specification, to block splicing and facilitate thiol cleavage. Such thiol cleavage replaces a nucleophilic attack on an acyl intermediate formed in the process of protein splicing between the target polypeptide and the carboxyl-proximal intein by the amino-terminal cysteine of another, more carboxyl-proximal extein. But it also leaves a free thioester at the carboxyl terminus of the accepting polypeptide making this "first target" protein of the claims vulnerable to attack by any free amine, such as those of lysine and arginine, which attack need not result in a peptide bond. To ensure formation of a peptide bond between first and second target proteins, Applicant's disclosed method provides the second "target" protein, the ligation partner introduced *in vitro*, with an amino-terminal cysteine which is sufficiently nucleophilic to preferentially attack the free thioester and form a peptide bond between the "first target", or accepting, polypeptide and the "second target", or splicing partner, polypeptide or peptide. As no accord was reached on further amendments after the June 20, 2002 interview, a new ground of rejection is stated under the first paragraph of 35 U.S.C. §112 and this communication is not made final.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. §112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-4, 6, 8-12, 15-19, 21, 24, 25, 27, 28, 31-45, 47 and 49-61 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

This is a new ground of rejection. The specification fails to exemplify or describe the practice of methods, or the preparation of starting products of the methods, or resulting products of the methods, of claims 1-4, 6, 8-12, 15-19, 21-25, 27, 28, 31-45, 47 and 49-61 wherein a "specified" - yet unspecified by, e.g., a Markush set, in claims 1, 8, 9, 16, 17, 24, 40, and 42 - amino-terminal amino acid of a ligation partner protein or peptide is a generic nucleophile. The specification discloses and suggests only cysteine as the amino-terminal amino acid of a ligation partner polypeptide or peptide and neither the claims nor the specification describe the design, preparation, or use in a method, of any nucleophile, nor a source thereof, that permits an amine-thiol acyl shift to form a peptide bond other than cysteine. "While one does not need to have carried out one's invention before filing a patent application, one does need to be able to describe that invention with particularity" to satisfy the description requirement of 35 U.S.C. §112, first paragraph. *Fiers v. Revel v. Sugano*, 25 USPQ2d 1601, 1605 (Fed. Cir. 1993). The specification furnishes no relevant identifying characteristics of any amino acid other than cysteine that serves as an amino-terminal amino acid of a ligation partner protein or peptide. Indeed, the specification does not "specify" or indicate which amino acids might be appropriate amino-terminal amino acid with a negative description, i.e., teaching (a) characteristic(s) that should be excluded or avoided in selecting an amino-terminal amino acid of a ligation partner polypeptide or peptide. Save for cysteine, the specification is silent about all other naturally-occurring and non-native amino acids at the amino-terminus of a ligation partner.

The Court of Appeals for the Federal Circuit held that a claimed invention must be described with such "relevant identifying characteristic[s]" that the public could know that the inventor possessed the invention at the time an application for patent was filed, rather than by a mere "result that one might achieve if one had made that invention". *University of California v. Eli Lilly*, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). Indeed, the claims rejected herein are, like the claims invalidated by the appellate panel in *University of California v. Eli Lilly*, designed to embrace other, as yet unknown, chemical species. Nothing demonstrates that Applicant was "able to envision" enough of the structure of any undisclosed, generic, amino acid at the time the specification was filed to provide the public with identifying "characteristics [that] sufficiently distinguish it . . . from other materials". *Fiers*, 25 USPQ2d at 1604 (citing *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991). The specification's treatment of the claimed subject matter is considered to be entirely prospective where skilled artisans in the relevant field of molecular biology could not recognize Applicant's possession in the disclosure of the knowledge of the structure, or other properties, of unspecified amino-terminal amino acids of a ligation partner polypeptide or peptide and claimed methods of use thereof.

The following is a quotation of the second paragraph of 35 U.S.C. §112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-4, 6-12, 14-21, 24, 25, 27, 28, 31-45 and 47-61 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is indefinite in reciting, in clause (a), "generating a first target protein . . . wherein the [first] target protein is expressed in a host cell" because it is incomplete and ambiguous in describing a disclosed method wherein a first target protein is expressed in a host cell but wherein a carboxyl-terminal thioester - the necessary factor for joining a

process of clause (a) with processes of clauses (b) and (c) - is not "generated" in a host cell. Indeed, the target protein is not the protein expressed in the host cell, but only a portion of that protein, and that portion can only become a "target" protein for ligation with a product of clause (b) outside the host cell when the precursor expressed as an extein-intein fusion is cleaved to form the "target" protein and a free intein. Claim 1 is further indefinite in reciting, in clause (b), "generating a second target protein having a specified N-terminal" because this description of a disclosed method wherein a second target protein is prepared with a certain amino-terminal amino acid is incomplete and ambiguous where only a single amino-terminal amino acid is disclosed to be suitable for ligation of a second target protein to a first target protein and there is no disclosure of any set, collection, or assortment, of "specified" amino acids that may be present at the amino terminus. This aspect of the rejection may be overcome by amending clause (a) of the claim to more carefully relate the expression of a first target protein and formation of a carboxyl-terminal thioester thereon, because recombinant expression alone cannot provide a carboxyl-terminal thioester, and by amending clause (b) of the claim to more carefully describe the requisite amino terminus of a second target protein. Clause (d) of the claim is also indefinite because clause (c) of the claim - simply combining the products described by clauses (a) and (b) - necessarily, and without more, results in ligation according to Applicant's disclosed method. Step (d) is thus superfluous where there is no separate step of "ligating" after combining the target proteins *in vitro*. This aspect of the rejection may be overcome by deleting clause (d) and by amending clause (c) to recite, "combining the first . . . in a mixture permitting ligation of the first and second target proteins." Claims 2, 3, 6, 7, 15 and 31-36 are subject to this rejection because they depend from claim 1 but do not resolve its ambiguities. The terminal recitation of claim 2, "intein having carboxyl-terminal activity", is also indefinite because it fails to identify the nature of the activity.

Claims 7, 14, 20, and 48 are independently rejected as indefinite for their recitations of the phrase, "comprises cysteine", in claims 7, 14 and 20 and recitation of the phrase, "comprises a cysteine", in claim 48. While an amino-terminal region of a polypeptide or peptide might properly be said to "comprise" an amino acid because there will be several amino acids in such a region, the amino terminus of a polypeptide or peptide must be a single amino acid. Neither terminus of a translated polypeptide or peptide can be multiple amino acids and the claims from which claims 7, 14, 20, and 48 describe or embrace the recombinant production of both target polypeptides or peptides, inherently requiring ribosomal translation of the targets. Thus, the recitation of "comprises" is illogical: an amino terminus is a single amino acid and a "specified N-terminal" must be a single amino acid. This aspect of the rejection may be overcome by amending claims 7, 14, 20 and 48 to delete "comprises" and to substitute "is" instead, as claim 26 currently recites.

Claim 8 is indefinite in reciting, "expressing at least one second target protein having a specified N-terminal", in clause (d) because the description is incomplete and ambiguous where only a single amino-terminal amino acid is disclosed to be suitable for ligation of a second target protein to a first target protein and there is no disclosure of any set, collection, or assortment, of "specified" amino acids that may be present at the amino terminus. This aspect of the rejection may be overcome by amending clause (d) of the claim to more carefully describe the requisite amino terminus of a second target protein. Claims 9-12, 15 and 37-39 are subject to this rejection because they depend from claim 8 but do not resolve its ambiguity.

Claims 16 and 17 are indefinite in reciting in clauses (b) of both claims, "expressing at least one second target protein having a specified N-terminal", because the description is incomplete and ambiguous where only a single amino-terminal amino acid is disclosed to be suitable for ligation of target proteins, one to another, and there is no disclosure of any

set, collection, or assortment, of "specified" amino acids that may be present at the amino terminus. This aspect of the rejection may be overcome by amending clauses (b) of these claims to more carefully describe the requisite amino terminus of a target protein that is a ligation partner. Claims 19-21 are subject to this rejection because they depend from claims 16 or 17 but do not resolve the ambiguity of clause (b) of these claims.

Claim 24 is indefinite in reciting, "to produce a specified residue at the N-terminal of an adjacent target protein", because the description is incomplete and ambiguous where only a single amino-terminal amino acid is disclosed to be suitable for ligation of target proteins, one to another, and there is no disclosure of any set, collection, or assortment, of "specified" amino acids that may be present at the amino terminus. This aspect of the rejection may be overcome by amending the claims to more carefully describe the requisite amino terminus of a target protein. Claims 25-28 are subject to this rejection because they depend from either of or both of claims 16 and 17 but do not resolve the ambiguity of clause (b) of these claims.

Claim 40 is indefinite in reciting, "applying means" in clause (a) because generation of the fusion proteins in the body of clause (a) requires the application of more than one means, rendering the claim an improper claim under the sixth paragraph of the statute, which states,

> An **element** in a claim for a combination may be expressed as a **means** or step for performing a **specified function** without the recital of structure, material, or acts in support thereof, and such claim shall be construed to cover the corresponding structure, material, or acts described in the specification and equivalents thereof.

(emphases supplied). Because the design and synthesis of encoding nucleic acids for both fusion proteins, the insertion of the encoding nucleic acids in expression plasmids, the transformation of host cells with the plasmids, and induction of expression of the fusion polypeptides by the host cells are all separate functions, they cannot constitute a unitary, specified, function that the sixth paragraph of the statute requires as a claim element,

rendering the claim ambiguous. Clause (b) of the claim is similarly indefinite where it inherently requires the application of separate means for two different processes mistakenly cast as a single element. While a "means" can properly be said to be applied "for cleaving the first . . . fusion protein" to provide an acceptor target protein - the specification describes several means for this - the means for cleavage of the first cannot be the same means applied for cleavage of the second fusion protein. In addition, while the second target protein must be cleaved from an adjacent intein of the fusion protein, it does not acquire "a specified N-terminal" through application of a "means for cleaving". Instead, the specification discloses that an amino-terminal cysteine is pre-existing, provided by the selection of a codon for cysteine in preparing the encoding nucleic acid sequence, a process embraced by clause (a) of claim 40. Claim 40 is further indefinite in reciting clause (c): there are no further "means applied" to permit ligation of the first and second target protein. Ligation occurs because the first and second target proteins already possess functional groups, one of which is recited in clause (b) of the claim, that mediate ligation without further intervention by a "means". Claim 40 is additionally indefinite in reciting, "a specified N-terminal on the second target protein", in clause (b) because this is incomplete and ambiguous where only a single amino-terminal amino acid is disclosed to be suitable for ligation of target proteins, one to another, and there is no disclosure of any set, collection, or assortment, of "specified" amino acids that may be present at the amino terminus. Claim 41 is subject to this rejection because it depends from claim 40 but does not resolve the ambiguity of that claim. Claim 41 is additionally indefinite because claim 40, from which it depends, provides no antecedent basis for the term "cleaved inteins" where such cleaved inteins may be cleaved anywhere within their amino acid sequence in addition to an extein-intein junction. Claim 40 must make reference to exteins to provide a definite description of the subject matter intended in the recitation of claim 41.

Claim 42 is indefinite in reciting, "so as to provide a specified N-terminal", in clause (b) because this is incomplete and ambiguous where only a single amino-terminal amino acid is disclosed to be suitable for ligation of target proteins, one to another, and there is no disclosure of any set, collection, or assortment, of "specified" amino acids that may be present at the amino terminus. Claims 43-45 and 49-57are subject to this rejection because they depend from claim 42 but do not resolve the ambiguity of clause (b) of the claim. Claim 59 is indefinite in reciting, "to remove the cleavage element", because the claim fails to indicate the nature of a "cleavage element" in phrase (i) and establishes no clear connection between removing an element and reaction with the reagent. Clause (ii) of the claim is superfluous: reaction with a thiol reagent inherently cleaves at an intein-extein junction and produces a carboxyl-terminal thioester. Claims 60 and 61 are subject to this rejection because they depend from claim 59 but fail to resolve its ambiguities.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claim 1 is rejected under the judicially created doctrine of double patenting over claim 96 of U.S. Patent No. 5,934,247 since the claim, if allowed, would improperly extend the "right to exclude" already granted in the patent. The subject matter claimed in the instant application is fully disclosed in the patent and is covered by the patent since the patent and the application are claiming common subject matter, as follows: a method of

synthesizing a desired protein, which includes the ligation of its components, wherein an expressed protein is joined by a peptide bond to another protein, which may be a peptide, wherein a first component protein has a carboxyl-terminal thiol ester, also known as a thioester, and a second target protein has a "specified" amino-terminal amino acid, a cysteine, which thioester and cysteine will form a peptide bond upon contact of the two component proteins with one another in solution *in vitro*.

Furthermore, there is no apparent reason why applicant was prevented from presenting a claim corresponding to claim 1 of the instant application during prosecution of the application that matured into the patent. See *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). See also MPEP § 804.

Claims 1, 15, 21, 32 and 59 are provisionally rejected under the judicially created doctrine of double patenting over claims 7, 12, 14, 16 and 22-24 of copending Application No. 09/786,009. This is a provisional double patenting rejection since the conflicting claims have not yet been patented. The subject matter claimed in the instant application is fully disclosed in the referenced copending application and would be covered by any patent granted on that copending application since the referenced copending application and the instant application are claiming common subject matter, as follows: 1) a cyclic protein produced by ligation of a protein having a carboxyl-terminal thioester and another protein, which may be a peptide, having an amino-terminal cysteine, 2) a method of making a protein having a carboxyl-terminal thioester by reacting an expressed precursor protein - which may be a fusion polypeptide comprising the desired polypeptide and an intein where the intein may be any derivative of an intein or any mutant thereof - with a thiol reagent to displace the intein and produce the carboxyl-terminal thioester, and 3) a general method of making any desired protein by ligating a portion of a recombinantly-expressed precursor protein upon which a carboxyl-terminal thioester is formed by reacting the precursor with a thiol reagent and then combining this desired portion with another desired portion have an amino-terminal cysteine.

Furthermore, there is no apparent reason why applicant would be prevented from presenting claims corresponding to those of the instant application in the other copending application. See *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). See also MPEP § 804.

Claim Rejections - 35 USC §103

The following is a quotation of 35 U.S.C. §103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR §1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. §103(c) and potential 35 U.S.C. §§102(f) or (g) prior art under 35 U.S.C. §103(a).

Claims 1 and 15 are for reasons of record rejected under 35 U.S.C. §103(a) as obvious over Canne et al., U.S. Patent No. 6,326,468.

Applicant's amendments to claim 1 do not avoid the teachings of Canne et al., see cols. 14-15, of the preparation of a protein wherein the mechanism of intramolecular acyl transfer is used to ligate a first target protein that is generated with a carboxyl-terminal thioester to a second target protein generated with a specified amino terminal amino acid, cysteine, to form a fusion polypeptide. While claim 1 now requires a first protein to be "expressed" "in a host cell" it does not require that a carboxyl-terminal thioester appear on this first, target, or accepting, protein as a result of thiol-mediated cleavage of an extein target from a carboxyl-proximal fused intein fragment, or even from an integral intein. Thus a protein of clause (a) of claim 1 may indeed be recombinantly expressed but be "generated" as a target protein by solid-phase addition of an amino acid having a carboxyl-terminal thioester where Canne et al. teach, col. 5, lines 27-31, that an accepting protein ligated by their method may be "recombinantly expressed".

Claim 1 and 15 are for reasons of record rejected under 35 U.S.C. §103(a) as obvious over Tam, U.S. Patent No. 6,310,180.

Applicant's amendments to claim 1 do not avoid the teachings of Tam, Figure 26 and discussion at cols. 36-42, of preparation of a protein wherein the mechanism of intramolecular acyl transfer is used to ligate a first target protein that is generated with a carboxyl-terminal thioester to a second target protein generated with a specified amino terminal amino acid, cysteine, to form a fusion polypeptide. While claim 1 now requires a first protein to be "expressed" "in a host cell" it does not require that a carboxyl-terminal thioester appear on this first, target, or accepting, protein as a result of thiol-mediated cleavage of an extein target from a carboxyl-proximal fused intein fragment, or even from an integral intein. Thus a protein of clause (a) of claim 1 may indeed be recombinantly expressed but be "generated" as a target protein by solid-phase addition of an amino acid having a carboxyl-terminal thioester where Tam teaches, col. 41, lines 54-57, that proteins to be ligated by the method include those available from, and "derived from . . . recombinant DNA methodologies".

Claim 1 and 15 are for reasons of record rejected under 35 U.S.C. §103(a) as obvious over Kent et al., U.S. Patent No. 6,184,344.

Applicant's amendments to claim 1 do not avoid the teachings of Kent et al., Figure 1 and discussion thereof and Examples 1-5 at cols. 11-16, of preparation of a protein wherein the mechanism of intramolecular acyl transfer is used to ligate a first target protein that is generated with a carboxyl-terminal thioester to a second target protein generated with a specified amino terminal amino acid, cysteine, to form a fusion polypeptide. While claim 1 now requires a first protein to be "expressed" "in a host cell" it does not require that a carboxyl-terminal thioester appear on this first, target, or accepting, protein as a result of thiol-mediated cleavage of an extein target from a carboxyl-proximal fused intein fragment, or even from an integral intein. Thus a protein of clause (a) of claim 1 may indeed be recombinantly expressed but be "generated" as a target protein by solid-phase

addition of an amino acid having a carboxyl-terminal thioester where Kent et al. teach, in the paragraph spanning cols. 8-9, that proteins to be ligated by the method include those "expressed from by standard rec[ombinant]DNA means".

Claim 1 and 15 are for reasons of record rejected under 35 U.S.C. §103(a) as obvious over Offord et al., U.S. Patent No. 6,168,784.

Applicant's amendments to claim 1 do not avoid the teachings of Offord et al., cols. 6-8 and cols. 11-14, of the preparation of a RANTES protein wherein the mechanism of intramolecular acyl transfer is used to ligate a first target protein that is generated with a carboxyl-terminal thioester to a second target protein generated with a specified amino terminal amino acid, cysteine, to form a fusion polypeptide. While claim 1 now requires a first protein to be "expressed" "in a host cell" it does not require that a carboxyl-terminal thioester appear on this first, target, or accepting, protein as a result of thiol-mediated cleavage of an extein target from a carboxyl-proximal fused intein fragment, or even from an integral intein. Thus a protein of clause (a) of claim 1 may indeed be recombinantly expressed but be "generated" as a target protein by solid-phase addition of an amino acid having a carboxyl-terminal thioester where Offord et al. teach, col. 7, lines 56-58, that proteins ligated by the method include those made "ribosomally in a cell free system, or ribosomally within a cell".

Claim 1 and 15 are for reasons of record rejected under 35 U.S.C. §103(a) as obvious over Hiatt et al., U.S. Patent No. 6,045,774.

Applicant's amendments to claim 1 do not avoid the teachings of Hiatt et al., col. 18, lines 7-26, the preparation of a transmembrane protein wherein the mechanism of intramolecular acyl transfer is used to ligate a first target protein that is generated with a carboxyl-terminal thioester to a second target protein generated with a specified amino terminal amino acid, cysteine, to form a fusion polypeptide. While claim 1 now requires a first protein to be "expressed" "in a host cell" it does not require that a carboxyl-terminal thioester appear on this first, target, or accepting, protein as a result of thiol-mediated

cleavage of an extein target from a carboxyl-proximal fused intein fragment, or even from an integral intein. Thus a protein of clause (a) of claim 1 may indeed be recombinantly expressed but be "generated" as a target protein by solid-phase addition of an amino acid having a carboxyl-terminal thioester where Hiatt et al. teach, cols. 19-20, lines 56-58, that the transmembrane proteins may be produced recombinantly and because one of ordinary skill in the art at the time the invention was made would have appreciated that component proteins of a transmembrane protein may also be made recombinantly and ligated by the method recognizing the advantage of doing so where smaller component proteins would be more easily isolated from prokaryotic host cells than an integral transmembrane protein.

Claims 2, 7-9, 14, 15, 31, 32, 36-43, 48, 50, 54-59 and 61 are, essentially for reasons of record, rejected under 35 U.S.C. §103(a) as being unpatentable over any of Canne et al., Tam, or Kent et al., as applied to claim 1 above, in view of Mills et al., 1998, **Proceedings of the National Academy of Sciences, USA**, Vol. 95, pages 3543-3548, of record.

Applicant's earliest priority document, utility application serial No. 08/811,492, has no disclosure corresponding to that of Mills et al., which was published before Applicant's other priority document, provisional application serial No. 60/102,413, was filed. The teachings of Canne et al., Tam, or Kent et al. are taken as before. While each of Kent et al., Tam and Canne et al. explicitly teach preparation of a desired, acceptor, polypeptide that will be ligated to a second, ligation partner, protein, where the carboxyl terminus of the acceptor protein provides a thiol ester for ligation with a second, ligation partner, protein having an amino-terminal cysteine, and while each generally teach that a protein destined for such ligation may be recombinantly produced, none of them explicitly teach the use of plasmid expression vectors and transformed host cells in recombinant expression of either ligation component wherein a plasmid expression vector comprises a nucleic acid sequence encoding a first target polypeptide fused at its carboxyl terminus to the amino terminus of an intein element. Mills et al. is therefore cited because they provide the

requisite motivation that one of ordinary skill in the art at the time the invention was made would have experienced to prepare a plasmid expression vector comprising a nucleic acid sequence encoding a first target polypeptide fused at its carboxyl terminus to the amino terminus of an intein element mutant intein in an *in vitro* method for trans-splicing. Mills et al. explicitly teach, see pages 3543 and 3544 and Fig. 8, that this may be induced by adding dithiothreitol [DTT] and raising the temperature of the *in vitro* solution to 25°C, whereby splicing provides a new fusion protein from a first recombinantly expressed target protein and a second recombinantly expressed target protein, and wherein the first target protein comprised an amino-proximal target protein fused to a carboxyl-proximal mutant intein and the second target protein comprised an amino-proximal mutant intein fused to a carboxyl-proximal target protein. Mills et al. teach, page 3548, that their "results show that the N- and carboxyl-terminal intein fragments essentially constitute a polypeptide ligase system that allows the *in vitro* ligation of any two proteins fused to such fragments".

It would have been obvious to one of ordinary skill in the art to replace a chemically-synthesized second target protein taught by Canne et al., Tam, or Kent et al. in an *in vitro* method for fusing first and second target proteins with a recombinantly-expressed second target protein expressed as a fusion of an amino-proximal mutant intein and a carboxyl-proximal target protein according to Mills et al. and has an amino-terminal cysteine, and to specifically express a first target protein of Mills et al. liberated by thiol reagent-induced cleavage from a fusion of an expressed amino-proximal target protein fused to a carboxyl-proximal intein and having a carboxyl-terminal thioester available for formation of a peptide bond with N-terminal cysteine of the second target protein. This is because Mills et al. teach that inteins present in separate fusion proteins should be excised *in vitro* to release target proteins for concurrent splicing *in vitro* to form a new fusion protein, and because Canne et al., Tam, and Kent et al. teach how such splicing may be conducted by

providing a first target polypeptide having a carboxyl-terminal thioester to permit formation of a peptide bond with a nucleophilic attack upon the thioester by an amino-terminal cysteine of the second target protein, and because Mills et al. teach that mutant inteins may be used to promote the *in vitro* splicing of two recombinantly expressed target proteins encoded by separate nucleic acid sequences as fusion proteins each comprising a mutant intein borne by plasmid vectors.

Allowable Subject Matter

Claims 22, 23, 29 and 30 are allowed. Claims are free of the prior art of record and would be allowable if rewritten to avoid the rejections above under the first and second paragraphs of 35 U.S.C. §112. Claims 7, 13, 26 and 46 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 703.308.0583. The examiner can normally be reached between 7:00AM-5:30PM EST on Mondays and Wednesdays, between 7:00AM-1:30PM EST on Tuesdays and Thursdays, and between 8:30AM and 5:00PM EST on Fridays. The examiner's direct FAX telephone number is 703.746.3169. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy can be reached at 703.308.3804. Further fax phone numbers for the organization where this application or proceeding is assigned are 703.308.4242 for regular communications and 703.308.0294 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703.308.0196.

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September 9, 2002

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